

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
10 June 2004 (10.06.2004)

PCT

(10) International Publication Number
WO 2004/048559 A1

(51) International Patent Classification⁷: C12N 9/02,
15/81, 1/19, C12P 7/06

(21) International Application Number:
PCT/EP2003/013231

(22) International Filing Date:
25 November 2003 (25.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0227435.5 25 November 2002 (25.11.2002) GB

(71) Applicant (for all designated States except US): FLUX-
OME SCIENCES A/S [DK/DK]; Danmarks Tekniske
Universitet, Bygning 223, Søtofts Plads, DK-2800 Lyngby
(DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BRO, Christoffer
[DK/DK]; Snogegårdsvej 2 st. th., DK-2820 Gentofte
(DK). REGENBERG, Brigitte [DK/DK]; Gammel
Kalkbraenderivej 8, 1. th, DK-2100 København (DK).

NIELSEN, Jens [DK/DK]; Hans Bruuns Vej 8, DK-2920
Charlottenlund (DK).

(74) Agent: SMART, Peter, J.; W.H. Beck, Greener & Co., 7
Stone Buildings, Lincoln's Inn, London WC2A 3SZ (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,
CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN,
MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU,
SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

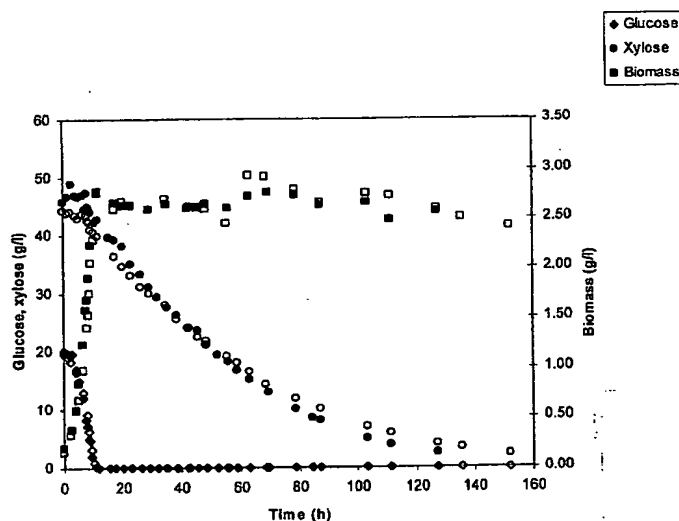
(84) Designated States (regional): ARIPO patent (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

[Continued on next page]

(54) Title: METABOLICALLY ENGINEERED MICRO-ORGANISMS HAVING REDUCED PRODUCTION OF UNDESIRE
METABOLIC PRODUCTS



(57) Abstract: A metabolically engineered micro-organism has an operative first metabolic pathway in which a first metabolite is transformed into a second metabolite in a reaction in which NAD is a cofactor for a first enzyme, suitably a phosphorylating dehydrogenase, said reaction step producing NADH. Said second metabolite is transformed into at least one further metabolite in a reaction catalysed by a second enzyme, suitably a kinase. The organism has an operative second metabolic pathway characterised by an enzyme activity in excess of a native level in respect of a third enzyme, suitably a non-phosphorylating dehydrogenase, e.g. GAPN, catalysing a non-reversible reaction in which NADP is a cofactor and NADPH is a product. Said first metabolite is transformed into a said further metabolite without the involvement of said second enzyme.

WO 2004/048559 A1



— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/13231

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/02 C12N15/81 C12N1/19 C12P7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>VALVERDE F ET AL: "Engineering a central metabolic pathway: glycolysis with no net phosphorylation in an Escherichia coli gap mutant complemented with a plant GapN gene"</p> <p>FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 449, no. 2-3, 23 April 1999 (1999-04-23), pages 153-158, XP004259550</p> <p>ISSN: 0014-5793</p> <p>cited in the application</p> <p>page 155, paragraph 1</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	<p>1-9, 13-16</p>



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

17 March 2004

Date of mailing of the international search report

02/04/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Aslund, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/13231

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>IDDAR ABDELGHANI ET AL: "Expression, purification, and characterization of recombinant nonphosphorylating NADP-dependent glyceraldehyde-3-phosphate dehydrogenase from Clostridium acetobutylicum"</p> <p>PROTEIN EXPRESSION AND PURIFICATION, vol. 25, no. 3, August 2002 (2002-08), pages 519-526, XP002273747</p> <p>ISSN: 1046-5928</p> <p>the whole document</p>	<p>1-9, 13-16</p>
A	<p>BIANCHI MICHELE M ET AL: "Efficient homolactic fermentation by Kluyveromyces lactis strains defective in pyruvate utilization and transformed with the heterologous LDH gene."</p> <p>APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 67, no. 12, December 2001 (2001-12), pages 5621-5625, XP002236026</p> <p>ISSN: 0099-2240</p> <p>the whole document</p>	<p>15</p>
A	<p>NIELSEN J: "Metabolic engineering."</p> <p>APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 55, no. 3, April 2001 (2001-04), pages 263-283, XP002236027</p> <p>ISSN: 0175-7598</p> <p>cited in the application</p> <p>the whole document</p>	<p>1-16</p>
A	<p>WANG Z ET AL: "Glycerol production by microbial fermentation - A review"</p> <p>BIOTECHNOLOGY ADVANCES, ELSEVIER PUBLISHING, BARKING, GB, vol. 19, no. 3, June 2001 (2001-06), pages 201-223, XP004255780</p> <p>ISSN: 0734-9750</p>	
A	<p>MICHNICK SUMIO ET AL: "Modulation of glycerol and ethanol yields during alcoholic fermentation in Saccharomyces cerevisiae strains overexpressed or disrupted for GPD1 encoding glycerol 3-phosphate dehydrogenase."</p> <p>YEAST, vol. 13, no. 9, 1997, pages 783-793, XP008015354</p> <p>ISSN: 0749-503X</p>	
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/13231

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VALADI H ET AL: "Improved ethanol production by glycerol-3-phosphate dehydrogenase mutants of <i>Saccharomyces cerevisiae</i> ." APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, vol. 50, no. 4, October 1998 (1998-10), pages 434-439, XP002236029 ISSN: 0175-7598 cited in the application ---	
A	ALEXANDRE H ET AL: "Global gene expression during short-term ethanol stress in <i>Saccharomyces cerevisiae</i> " FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 498, no. 1, 1 June 2001 (2001-06-01), pages 98-103, XP004243333 ISSN: 0014-5793 ---	
A	RODRGUEZ-ACOSTA F ET AL: "Non-linear optimization of biotechnological processes by stochastic algorithms: Application to the maximization of the production rate of ethanol, glycerol and carbohydrates by <i>Saccharomyces cerevisiae</i> " JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 68, no. 1, 5 February 1999 (1999-02-05), pages 15-28, XP004157315 ISSN: 0168-1656 ---	
A	NISSEN T L ET AL: "Optimization of ethanol production in <i>Saccharomyces cerevisiae</i> by metabolic engineering of the ammonium assimilation." METABOLIC ENGINEERING. UNITED STATES JAN 2000, vol. 2, no. 1, January 2000 (2000-01), pages 69-77, XP002236030 ISSN: 1096-7176 -----	